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Conformation of brain proteolipid apoprotein

Effects of sonication and *n*-octyl-β-D-glucopyranoside detergent

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Abstract. The conformation of brain proteolipid apoprotein (PLA) has been investigated using infrared spectroscopy and freeze-fracture electron microscopy. For this purpose, spectroscopic samples consisting of a mixture of liquid paraffin and wet protein have been prepared. These systems have allowed us to record the infrared spectra of PLA at neutral pH. The amide I and III regions reveal the existence of a predominantly α-helical structure, as well as the presence of minor β-strands and random coil forms. The effect of sonication and a non-denaturing detergent, (*n*-octyl-β-D-glucopyranoside), on the structure of the protein have also been investigated. Sonication produces an increase of the β and unordered structures at the expense of the α-helical conformation. These structural changes are enhanced in the presence of the non-ionic detergent *n*-octyl-β-D-glucopyranoside. Lipids protect the native protein structure from the effects of sonication. The aforementioned detergent changes the PLA conformation by increasing the α-helical content at the expense of β-sheet and random coil forms. Therefore the PLA structure seems to be similar to the structures of other proteins intrinsic to non-neural membranes. The effects investigated also suggest that PLA behaves in a conformationally flexible manner.

Key words: Proteolipid apoprotein structure, sonication, non-denaturing detergent, freeze-fracture electron microscopy, infrared spectra

Introduction

The brain is one of the organs in mammals containing the highest proportion of membranes. The study of its components is important in the understanding of the structure and function of the nervous system. Despite the significant biological role of brain membranes, knowledge of their protein and lipid content is incomplete.

The myelin membrane is one of the main components of brain white matter and PLP (proteolipid protein) is the major protein constituent which represents more than 50% of the total protein. Proteolipid protein is a mixture consisting of about 40% protein and 60% phospholipids and glycolipids. More than 50% of its amino acids are hydrophobic and interact strongly with the mixture of phospholipids. This protein is also covalently bound to about 2.5% of fatty acids. Proteolipid apoprotein (PLA) is the delipidated form of PLP and is a good model for the study of lipid-protein interactions (Cozar 1987; Abney and Owicki 1985). Freeze-fracture electron micrographs of myelin and of recombinants of PLA with dimiristoylphosphatidylcholine (DML) (Massa and Mugnaini 1982; Garcia-Segura et al. 1986) show particles in the fracture plane, characteristic of proteins deeply embedded in the hydrocarbon region of the bilayer. Recent membrane studies suggest that proteolipid protein may act as an ionophore (Waxman and Ritchie 1985; Cozar et al. 1987b).

Due to its great hydrophobicity and the lack of lipids, PLA forms strong aggregates that are difficult to solubilize in the presence of non-ionic detergents (Crang and Rumsby 1978; Aguilar et al. 1982; Smith et al. 1984; Moriyama and Makinos 1985). Sonication, a widly used technique, can be used to break these aggregates (Mendelsohn et al. 1976; Bakouche et al. 1986; Furia et al. 1986; Lin et al. 1986; Zasadzinski 1986). The effect that sonication has on the conformational state of PLA has, however, not yet been studied. The influence that detergents have on the conformation of these proteins is also not well known. These factors have made the investigations of the conformational state of PLA difficult.

We have investigated at neutral pH the effect of the non-denaturing detergent, *n*-octyl-β-D-glucopyranoside, that does not interfer spectroscopically with the

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amide I region of proteins nor with the conformation of PLA. Infrared spectroscopy is a powerful non-perturbing technique that has been applied very recently to determine the secondary structure of myelin (Ayala et al. 1987). We have not found any report concerning the conformational state of PLP using infrared and Raman spectroscopy. Cockle et al. (1978) studied PLA in 2-chloroethanol and phenol-acetic acid-urea mixtures using circular dichroism in order to determine the conformation of PLA suspended in different solvent conditions. The protein conformation of PLA was found to be dependent on the solvent mixture used.

In this paper we have demonstrated the incorporation of wet PLA into liquid paraffin using freeze-fracture electron microscopy. The conformation of PLA under these conditions, which mimic the biological membranes, has also been investigated. We have obtained infrared spectra of PLA in liquid paraffin that gives to the system the appropriate hydrophobic environment. On the other hand, wet protein samples have enabled us to study this system with the appropriate hydrophilic medium. The above described conditions have permitted us to determine the conformational state of PLA and the influence of sonication and the detergent *n*-octyl-*β*-D-glucopyranoside on the structure of this protein.

Experimental

Preparation of PLP and PLA

Beef brains were obtained from recently slaughtered animals and immediately transported to the laboratory in crushed ice.

PLP was purified from brain white matter, lyophilized and subsequently stored at −30 °C (Monreal 1975; Aguilar et al. 1982). These samples contain protein (40%) and phospholipids (60%).

PLA preparation was carried out by delipidation of PLP using ultrafiltration (Aguilar et al. 1983). With this procedure more than 99% of phospholipids were removed.

Freeze-fracture electron microscopy

Sonicated samples of PLA and PLP were suspended in Nujol (liquid paraffin, spectroscopic grade, Merck), fixed in 1% paraformaldehyde and 1% glutaraldehyde in 0.1 *M* phosphate buffer, pH 7.4 at 22 °C for 1 h, washed several times in 0.1 *M* phosphate buffer, pH 7.4, then soaked for 2 h in the same buffer containing 20% glycerol and frozen in Freon 22 previously cooled with liquid nitrogen. The samples were fractured and shadowed with platinum/carbon at −110 °C in a Balzers 400 D apparatus (Balzers, Liechtenstein). Freeze-fracture replicas wer photographed in a Jeol 100 B electron microscope.

Sonication

A B-12 Branson sonicator (Sonic Power, Connecticut, USA) was used. A micro tip was used for the treatment of ice cooled samples (20 mg of lyophilized PLA or 50 mg lyophilized PLP, equivalent to 20 mg of protein) in 1 ml. Sonication was carried out for 30 s (2 × 15″) at 60 W of power output.

Spectroscopy

Lyophilized PLP or PLA samples were suspended either in 0.1 *M* phosphate buffer (pH 7.4) or in the same buffer containing 150 m*M* *n*-octyl-*β*-D-glucopyranoside. The two deuterated buffers were prepared at the same phosphate and detergent concentrations using D_2O.

Deuterated samples were prepared as follows: 10 mg of PLA or 25 mg of PLP (equivalent to 10 mg of protein) were suspended in 0.5 ml of D_2O and immediately sealed and keeped inside a desiccator for 2 h, followed by lyophilization. These deuteration processes were then repeated. The lyophilized proteins were then resuspended in 0.5 ml of the deuterated buffers and an additional incubation of 2 h was carried out before scanning the infrared spectra. Deuteration was checked by means of the amide I and II bands. The band intensity ratio of amide II/amide I decreased in deuterated samples as a result of the isotopic substitution of the peptide band N–H to N–D, representing exchanges of 70%–80% of these hydrogens.

We have studied the effects of sonication and of the presence of *n*-octyl-*β*-D-glucopyranoside using heavy water to avoid the influence of the H_2O deformation band on the amide I region that is relied on for the characterization of the PLA conformation.

Each of the samples described were divided in two portions (only one was sonicated) and their spectra were recorded.

Two or 3 mg of the wet protein precipitates were homogenized in liquid paraffin (Nujol, Merck) and were then placed between CaF_2 windows with a mean layer thickness of 10 μm. These Nujol mulls were prepared inside a glove box to protect the deuterated samples from H, D exchange with the atmospheric H_2O. These Nujol mulls constitute the samples whose infrared spectra are referred to as original spectra in the caption of the figures. A Nujol mull containing only the deuterated buffer was prepared in a similar way and its infrared spectrum was substracted from the original spectra. The resulting difference spectra, which are shown in the figures, wer obtained using the

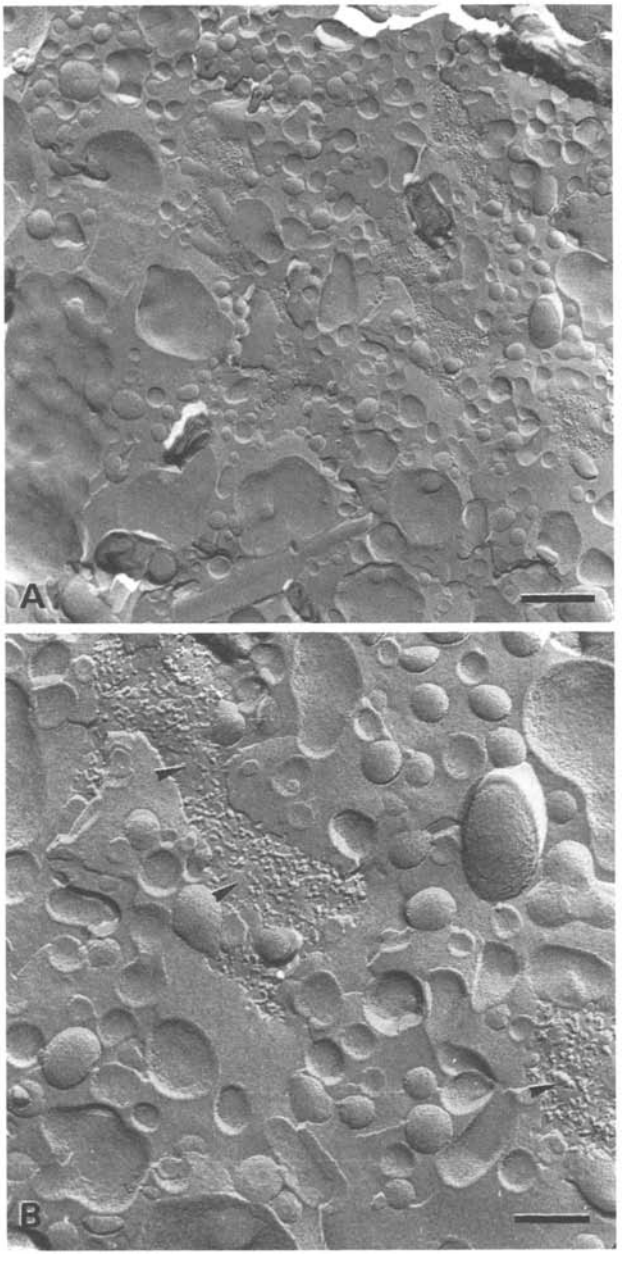

Fig. 1. Panoramic view (**A**) and high magnification (**B**) of a freeze-fracture replica of a preparation of PLP samples suspended in Nujol, showing aggregates of intramembrane particles (see *arrows*). **A** *bar* = 0.4 μm. **B** *bar* = 0.2 μm

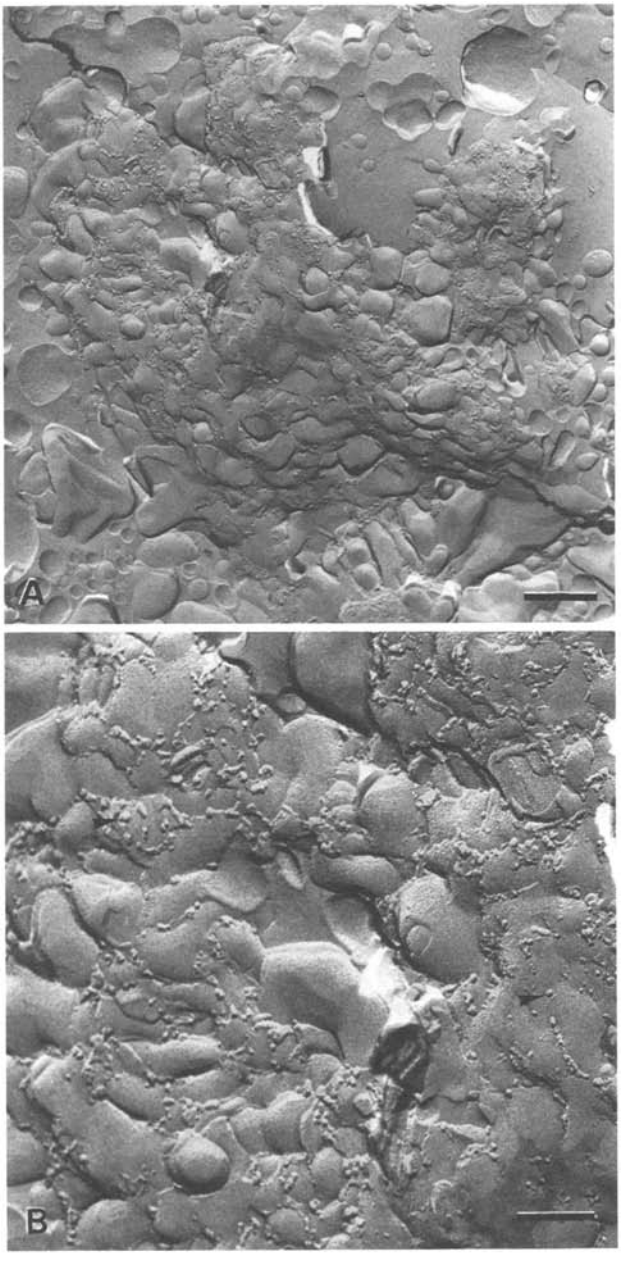

Fig. 2. Panoramic view (**A**) and high magnification (**B**) of a freeze-fracture replica of a preparation of PLA samples suspended in Nujol. Intramembrane particles are dispersed in the liquid paraffin (see *arrows*). **A** *bar* = 0.4 μm. **B** *bar* = 0.2 μm

SDIFF subroutine of a data station (Perkin Elmer, model 3600) connected to an infrared spectrophotometer (Perkin Elmer, model 599 B). The spectra of the mulls containing either the protein wet precipitate or the deuterated buffer were recorded using the same instrumental conditions. Five scans at 2 cm^{-1} resolution were averaged (Ayala et al. 1987). Second-derivative spectra were generated from difference spectra by means of an Obey program (Perkin-Elmer).

Results and discussion

1. Freeze-fracture electron microscopy

Intramembrane particles (IMP), that correspond to proteins in the fracture plane, were observed in the freeze-fracture replicas of PLP and PLA samples (Figs. 1 and 2). The distribution of IMP was different in PLP samples when compared to PLA preparations. In PLP samples two domains were observed: one

formed by aggregates of IMP packed at a high density and the other in which IMP were excluded (Fig. 1). In PLA samples IMP were dispersed in the liquid paraffin phase (Fig. 2). This suggests that PLA is more easily embedded in liquid paraffin than PLP.

2. General assignment of amide bands and conformation of PLA

In the infrared spectra of proteins, the secondary structure is most clearly reflected by the amide I, II and III bands, particularly the first, which absorbs around 1620 to 1690 cm^{-1} and is primarily associated with the stretching vibrations of peptide carbonyl groups.

The amide I region in the infrared spectrum of undeuterated PLA exhibits an absorption maximum at 1652 cm^{-1} (Fig. 3, Table 1). Many proteins show a band near this frequency associated with helical segments in the polypeptide backbone (Olinger et al. 1986; Byler and Susi 1986). Part of the absorption of this amide I band component may also be due to random coil structure because this polypeptide arrangement causes an amide I component around 1655 to 1660 cm^{-1} (Olinger et al. 1986; De Lozé et al. 1978; Lord and Mendelsohn 1981). This overlapping can be resolved after deuterium exchange as described below. The position of the amide I′ absorption maximum at about 1652–1650 cm^{-1} (Table 2) (Olinger et al. 1986) suggests that the α-helical conformation is the predominant protein secondary structure in PLA. This is also supported by our preliminary Raman spectroscopic results that show a splitting between the infrared (1652 cm^{-1}) and Raman (1658 cm^{-1}) frequencies of the amide I band. It is well known that this splitting indicates the existence of an α-helical polypeptide arrangement (De Lozé et al. 1978; Ayala et al. 1987). Our infrared spectrum of PLA shows a weak absorption at 1240 cm^{-1} (Fig. 4), and Koenig and Tabb (1980) also reported weak amide II infrared bands near 1240 cm^{-1} for two helical proteins.

The second derivative spectrum of undeuterated PLA (Fig. 3) also shows amide I band components at 1690, 1685, 1674, 1666, 1635 and 1627 cm^{-1}. The presence of amide I components at 1690, 1635 and 1627 cm^{-1} strongly suggests that some β-structure is also present. The amide I component at 1685 cm^{-1} cannot be unambiguously assigned, as absorption near this frequency has been reported for turns and β-strands (Susi and Byler 1983; Olinger et al. 1986). Protein chains of undefined structure, turns and amino acid side chains are likely, according to literature reports (Olinger et al. 1986; Krimm and Bandekar

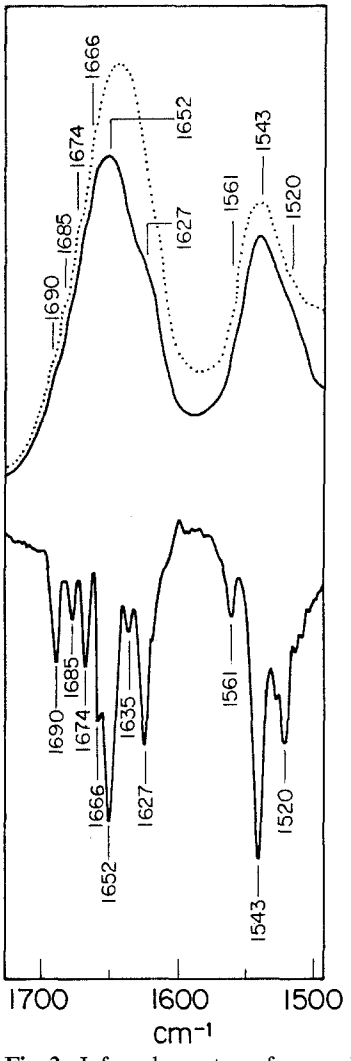

Fig. 3. Infrared spectra of normal (undeuterated) PLA suspended in Nujol. *Upper*: (*dotted line*) original and (*full line*) difference spectra. *Lower*: Second derivative of the difference spectrum, smoothed

Table 1. Infrared bands of normal (undeuterated) proteolipid apoprotein (PLA)

cm^{-1}	Assignment
1690 sh	Amide I (β-strands)
1685 sh	Amide I (turns and/or β-strands)
1674 sh	Amide I (turns and/or amino acid side chains)
1660 sh	Amide I (unordered)
1652 s	Amide I (α-helix)
1635 sh	Amide I (β-strands)
1627 sh	Amide I (β-strands)
1561 vw	Glutamic and aspartic acid residues
1543 s	Amide II (α-helix, unordered)
1520 sh	Amide II (β-strands and tyrosine side chain)
1305 w	$t(CH_2)$, $w(CH_2)$
1240 w	Amide III
1162 w	
1102 sh	$t(NH_2)$, $r(NH_3^+)$
1080 m	$\nu(C-C)$, $\nu_s(PO_2^-)$
1028 m	Phe

Abbreviations: s, m, w, vw: strong, medium, weak and very weak intensity; Phe, phenylalanine.

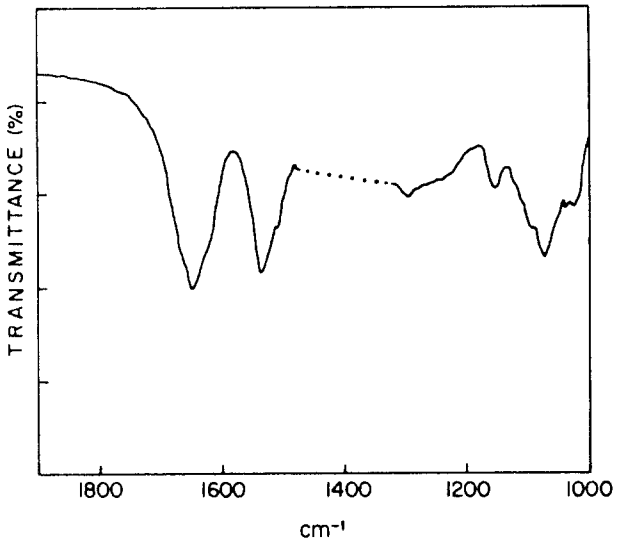

Fig. 4. Infrared spectrum of normal (undeuterated) PLA suspended in Nujol

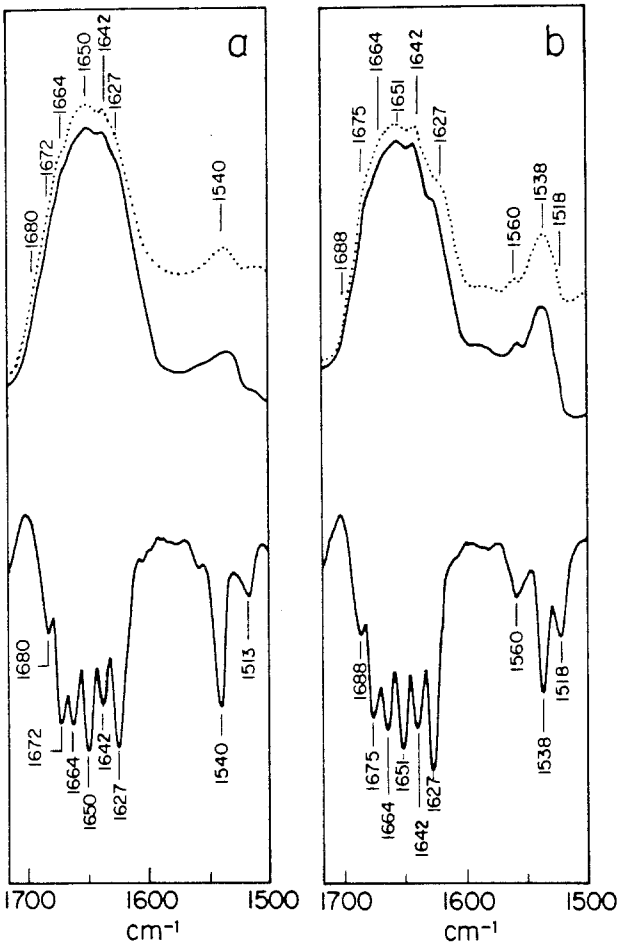

Fig. 5a and b. Infrared spectra of *deuterated* samples of (**a**) unsonicated PLA and (**b**) sonicated PLA in the *absence* of detergent. *Upper*: (*dotted line*) Original and (*full line*) difference spectra. *Lower*: Second derivative of the difference spectrum, smoothed. Samples suspended in Nujol

1986; Ruegg et al. 1975), to contribute to the band at 1674 cm^{-1}. The second derivative spectrum of this protein also exhibits a band at 1666 cm^{-1} which can be assigned to turns and/or unordered structures (Byler and Susi 1986; Olinger et al. 1986; Bandekar and Krimm 1980).

A large band centered at 1543 cm^{-1} and a shoulder at 1520 cm^{-1} correspond to the amide II region of the infrared spectrum (Fig. 3). The intensities of these two bands decrease upon deuteration and therefore can be assigned to amide II modes. The 1520 cm^{-1} shoulder can be attributed to β-strands on the basis of spectroscopic correlations of amide II frequencies with protein secondary structures (Parker 1971). However, part of the absorption of this shoulder also corresponds to tyrosine side chain absorption (Tu 1982; Chirgadze et al. 1975).

The amide I′ region in the second derivative spectrum of deuterated PLA (Fig. 7a) shows band components at 1652 and 1642 cm^{-1}. These frequencies indicate that these band components are due to α-helix and unordered structures respectively (Olinger et al. 1986; Byler and Susi 1986). The 1652 cm^{-1} band component (Fig. 3) does not shift upon deuteration (Fig. 7a) which contrasts with the shifts of 2–4 cm^{-1} to lower frequency reported for soluble α-helical proteins (Susi et al. 1967; Olinger et al. 1986). However, a similar case has recently been reported for the α-helical erythrocyte glucose transporter (Alvarez et al. 1987). Other amide I band components appear at 1680 and 1628 cm^{-1} suggesting the existence of β-strands as described above. Myelin membranes and PLP also contain the above-mentioned structures (Ayala et al. 1987).

Figures 7a and 8a and Table 2 show that PLP presents more helical conformation and less beta and random coil structures than PLA. This behaviour suggests a flexible conformational nature for PLA which is related to the proportion of lipids (Moscarello et al. 1973).

3. *Effects of sonication on the protein structure*

3.1. In the absence of detergent. Figures 5a, b and 6a, b show the spectra of PLA and PLP, both sonicated and unsonicated. These spectra show that sonication causes an increase of β-structure (1627 cm^{-1}) and unordered forms (1642 cm^{-1}) at the expense of α-helical structure (1651 cm^{-1}). These relative changes are clearly observed in the second derivative spectra and seem to be greater in PLA than in PLP. Lipid molecules seem to protect the protein structure against the effect of sonication in PLP (Fig. 6).

3.2. In the presence of detergent. The second derivative spectrum of deuterated PLA upon sonication, in the

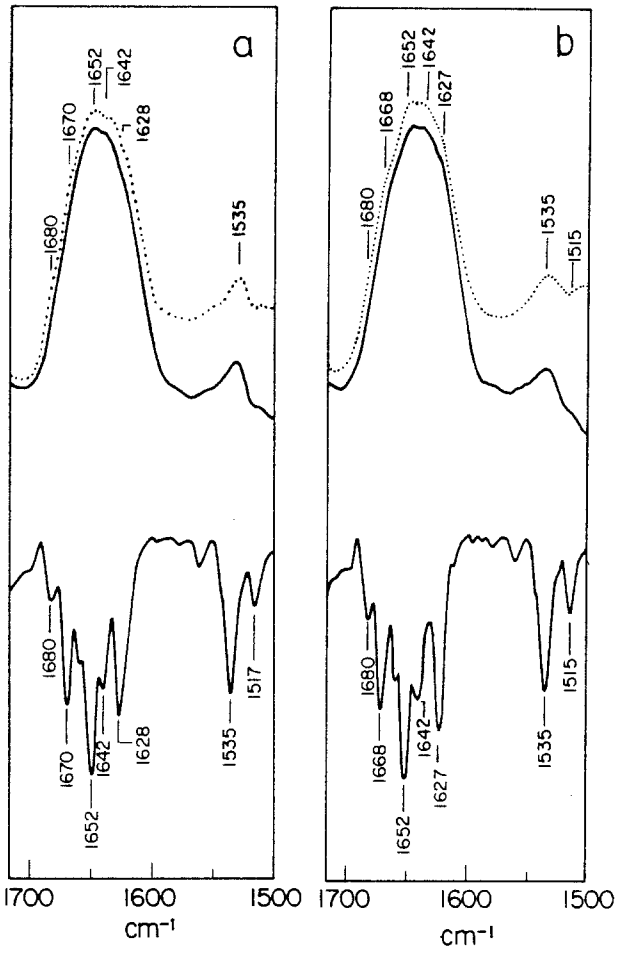

Fig. 6a and b. Infrared spectra of *deuterated* samples of (**a**) unsonicated PLP and (**b**) sonicated PLP in the *absence* of detergent. *Upper*: (*dotted line*) Original and (*full line*) difference spectra. *Lower*: Second derivative of the difference spectrum, smoothed. Samples suspended in Nujol

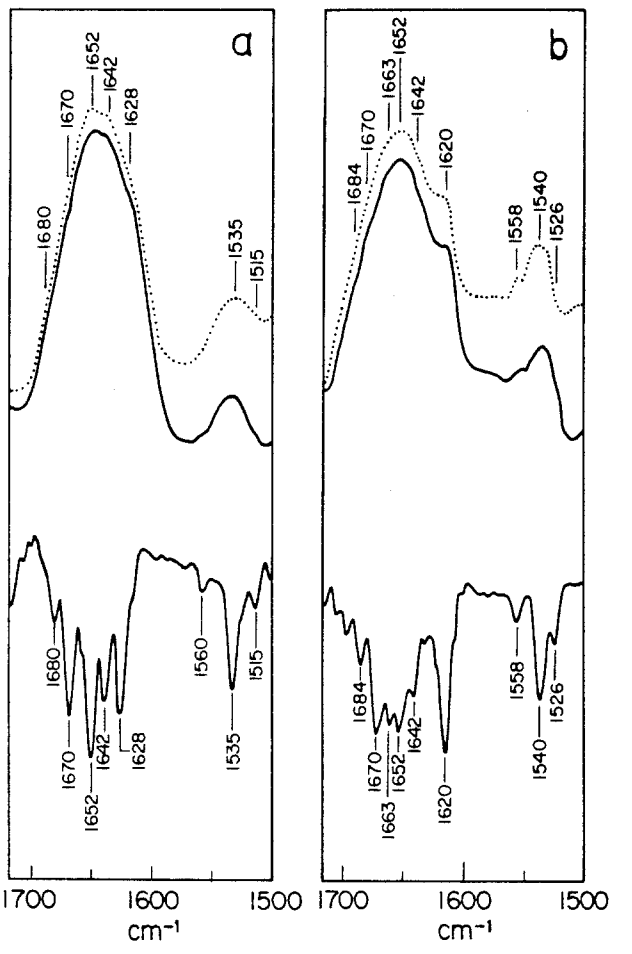

Fig. 7a and b. Infrared spectra of *deuterated* samples of (**a**) unsonicated PLA and (**b**) sonicated PLA in the *presence* of detergent. *Upper*: (*dotted line*) Original and (*full line*) difference spectra. *Lower*: Second derivative of the difference spectrum, smoothed. Samples suspended in Nujol

presence of detergent (Fig. 7b), reveals a relative decrease of the absorption maximum at 1652 cm^{-1} when compared with the spectrum of unsonicated PLA (Fig. 7a). In addition, relative increases of the band components of β-strands (1620 cm^{-1}), random coil (1642 cm^{-1}) and turns (1663, 1670 cm^{-1}) with respect to the α-component (1652 cm^{-1}) are also observed. On the basis of the spectroscopic assignment described above one can note that sonication increases the β-strands and unordered conformations at the expense of the α-helical arrangements, which causes an increase in the halfbandwidth of the amide I′ band. This is due to the presence of β-structure and to an increased halfbandwidth of random coil conformations (Table 2).

Similar effects are produced when PLP is sonicated, but the change in the spectral profile of this amide I′ region is less intense (Fig. 8, Table 2). This can be explained as follows: protein aggregation is associated

Table 2. Halfbandwidths (cm^{-1}) and frequencies of amide I′ bands of deuterated samples

	Without detergent				With detergent			
	Sonicated		Unsonicated		Sonicated		Unsonicated	
PLA		1688 sh		1680 sh		1684 sh		1680 sh
		1675 sh		1672 sh		1670 sh		1670 sh
	(79)	1664 sh	(77)	1664 sh	(74)	1663 sh	(69)	1663 sh
		1651 s		1650 s		1652 s		1652 s
		1642 s		1642 s		1642 sh		1642 sh
		1627 sh		1627 sh		1620 sh		1628 sh
PLP		1680 sh		1680 sh		1690 sh		1690 sh
		1688 sh		1670 sh		1672 sh		1688 sh
	(67)	1661 sh	(66)	1662 sh	(67)	1660 sh	(65)	1660 sh
		1652 s		1652 s		1651 s		1652 s
		1642 s		1642 s		1644 sh		1642 sh
		1627 sh		1628 sh		1628 sh		1628 sh

The values in parenthesis correspond to the halfbandwiths of the total amide I′ band. The shoulder positions are determined from the second derivative spectra

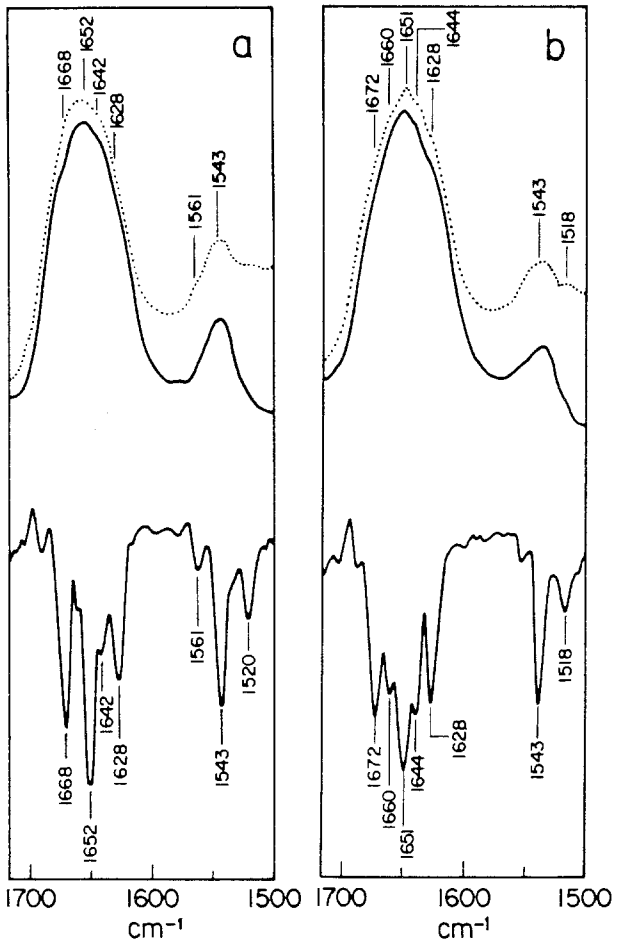

Fig. 8a and b. Infrared spectra of *deuterated* samples of (**a**) unsonicated PLP and (**b**) sonicated PLP in the *presence* of detergent. *Upper*: (*dotted line*) Original and (*full line*) difference spectra. *Lower*: Second derivative of the difference spectrum, smoothed. Samples suspended in Nujol

with an increase of intermolecular peptide bonds involved in β-sheet and random coil conformations (Levitt and Chothia 1976; Frushour and Koenig 1975). Since the proportion of lipids in PLA is very small and lipids in PLP are present in the proportion of 60%, it is evident that lipids protect the protein structure against sonication.

The fact that the effects of sonication are greater in the presence of the detergent than in its absence, can be explained by considering that the absence of the detergent produces an increase in PLA aggregation, whereby its polypeptidie chains are more tightly bound. Consequently, the penetration of sonication waves into the interior of protein aggregates becomes more difficult, and only the surface of aggregates exposed to direct sonication waves are modified. By contrast, the presence of the detergent disrupts PLA aggregates allowing the sonication waves to penetrate more easily into the aggregates and therefore modify the protein structure.

In relation to PLP, lipidic molecules also seem to protect the protein structure against the effect of sonication (Fig. 8 b, Table 2). The solubilizing effect on lipids of the detergent reduces the lipidic protection against sonication. For this reason, sonication has less effect on PLP structure in the absence of detergent than in its presence.

4. Influence of n-octyl-β-D-glucopyranoside on the conformation of PLA

The influence of *n*-octyl-β-D-glucopyranoside on the conformation of unsonicated PLA (Figs. 7 a and 8 a, Table 2) has also been investigated. Spectroscopic results reveal that the detergent increases α conformation and decreases the width of amide I band.

Membranes proteins are known to increase their α-helical content in a hydrophobic medium (Boggs and Moscarello 1978), therefore the helical conformation is a result of the burying of peptide groups in the hydrophobic core. The addition of the detergent contributes to this effect.

From the above mentioned results we can conclude: PLA, from a conformational point of view, exhibits a structure typical of intrinsic membrane proteins. The frequencies and intensities of the amide I and III bands are characteristic of a predominantly α conformation, with minor amounts of β and random coil conformations. The conformation of PLA is similar to that of PLP (Ayala et al. 1987) except that it has a greater content of β and random coil conformations. The protein adapts its structure according to lipid concentration. Sonication of PLA and of PLP produces alteration of the conformational states with an increase in β and random coil structures at the expense of the α structure. These alterations are greater in PLA and greater in the presence of detergent. Lipids, besides creating a hydrophobic environment appropriate to the protein, also exhibit a protecting effect on the protein structure, possibly through a shielding effect. Besides facilitating the effect of sonication the detergent also produces an increase of the α content at the expense of β and random coil structure, which mimics the mechanism of lipid interaction with the protein structures (Hellenius et al. 1979). These results suggest that the PLA structure is similar to the other intrinsic protein structures of non-neural membranes (Goldstein et al. 1986). The effects investigated also suggest that PLA behaves in a conformationally flexible manner (Moscarello et al. 1973). In order to estimate the conformational changes in the secondary structure of PLA, experiments of deconvolution of the Amide I band are in progress.

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